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CUTANEOUS TOXICITY EVALUATION OF AIR FORCE DEVELOPMENT MATERIALS – VII

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INDUSTRIAL BIOLOGY RESEARCH AND TESTING LABORATORIES, INC.

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AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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MORRIS V. SHELANSKI, MD

FOREWORD

This report was initiated by the Toxic Hazards Branch, Physiology Division, Biomedical Laboratory of the Aerospace Medical Research Laboratories. The contract monitor was Dr. Kenneth C. Back. The original research and development work upon which the report is based was accomplished by Industrial Biology Research and Testing Laboratories, Inc., 22 N. 36th Street, Philadelphia, Pennsylvania under Air Force Contract No. AF 33(615)-1571, in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630201, "Toxicology." The author, Dr. Morris V. Shelanski, was project director in charge of the basic research and development work. Research was begun in June 1964 and completed in October 1964. Mr. Hyman R. Gittes, Toxicologist, and Dr. Theodore Levenson, Chemist, cooperated in the research.

This is the seventh in a series of reports, entitled "Cutaneous Toxicity Evaluation of Air Force Development Materials," by the Industrial Biology Research and Testing Laboratories, Inc. The previous reports are:

I. WADC TR 56-626, December 1956, by M. V. Shelanski and C. Josephs

II. WADC TR 57-742, November 1957, by M. V. Shelanski and K. L. Gabriel

III. WADC TR 59-124, June 1959, by M. V. Shelanski and K. L. Gabriel

IV. ASD TR 61-77, April 1961, by M. V. Shelanski and K. L. Gabriel

V. MLR-TDR-62-26, April 1962, by M. V. Shelanski and K. L. Gabriel

VI. AMRL-TDR-64-13, February 1964, by M. V. Shelanski

This technical report has been reviewed and is approved.

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Technical Director
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ABSTRACT

Five Air Force development materials were studied via the prophetic patch test method on laboratory animals to determine the primary irritant effect, gross sensitization index, and gross percutaneous toxicity of these materials. The patch test studies with rabbits indicated that two of the materials produced severe primary irritant action. A third material produced primary irritation whose severity was not considered sufficient to preclude testing in humans. Testing on human volunteers was therefore carried out with three of the materials. Results indicated that only one of these materials was safe to use in contact with human skin.

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INTRODUCTION

Industrial Biology Research and Testing Laboratories, Inc., was engaged by the United States Air Force to perform dermatological studies and provide cutaneous toxicity data on certain Air Force development materials. These data would serve the Air Force as criteria for establishing safe handling procedures and limits of application of these materials when utilized by Air Force personnel.

There are various methods used for the determination of cutaneous toxicity of a chemical compound or substance. Laboratory animals, such as rabbits or guinea pigs, have been used by many investigators (ref. 1). The true index of cutaneous reaction can, however, only be determined by using human subjects. Prophetic patch tests are one of the methods used for this purpose (refs. 2 & 3). This test method helps to establish both the primary irritation and sensitization characteristics of a compound brought into contact with the human skin. Prophetic patch test studies were performed on laboratory animals to screen the primary irritant and sensitization characteristics of certain Air Force development materials. The Shelanski repeated insult patch test (ref. 3), in addition to giving information about primary irritation and sensitization characteristics of the compound, will also bring out any fatiguing reactions which may occur on continuous contact of the material with the human skin. This technique was performed on volunteer human subjects to define the characteristics of these compounds on the skin of humans.

MATERIALS

The following materials were received from the Aerospace Medical Research Laboratories:

1. Polacoat material PP 2038 Solution A, consisting of:

    a. 1,5-bis [4-(N, N-dimethylamine) phenyl] -1,5-bis (p-methoxyphenyl) divinyl carbonium lenco hydroxide - 8.7% by weight

    b. 2(2-methoxyethoxy) ethanol - 91.3% by weight

2. Polacoat material PP 2038 Solution B, consisting of:

    a. Bromoform - 72.0% by weight

    b. Dimethyl Sulfoxide - 28.0% by weight

3. Lubricating oil, Weapons MIL-L-14107

4. Molybdenum disulfide, technical grade, MIL-M-7866A(ASG)

A fifth material was compounded for testing by mixing equal volumes of Polacoat material PP 2038 Solution A and Solution B.

CRITERIA

FOR GRADING PATCH TEST REACTIONS

The investigators have discussed the criteria for grading patch test reactions used by various authors in a previous report, March 1955 (ref. 4). In this study, as in the previous, the following criteria were used by the Industrial Biology Research and Testing Laboratories, Inc.:

0 - no reaction, or questionable reaction

1+ - definite or clear-cut erythema

2+ - marked erythema, greater than present in 1+ reaction

3+ - marked erythema, edema, with or without a few vesicles

4+ - marked erythema, edema, with vesicles and oozing

RABBIT SCREENING STUDIES

PROCEDURE

Five groups of five albino rabbits each were used in this study. The animals selected weighed approximately two kilograms each. Prior to use, the animals were placed on colony diet and observed for a period of two weeks. Animals not showing normal weight gain were replaced.

Prior to patching, the fur on the back of each rabbit was closely clipped to expose an area of skin equal to at least 10% of the total body area. This area was then shaved to denude the skin completely. The patch site area was marked with permanent ink to identify the site for later reference.

The test materials were applied to the denuded skin, covered with glassine paper, and held in place by means of a muslin binder. Approximately four grams of each material was spread over the exposed area of skin for each application. Five rabbits per material were used. The first or primary application remained in contact with the denuded skin for forty-eight hours. Upon removal, reactions were graded and recorded. Twenty-four hours after removal of the patches, the sites were examined for delayed reactions.

Following the primary application, the animals were rested for fourteen days. The patch material was then reapplied on the same site as a challenge or sensitization application. Again, after forty-eight hours contact, the patches were removed and reactions graded and recorded. Twenty-four hours later, the sites were examined for delayed reactions. In the case of Polacoat material PP 2038 Solution B and the mixture of Polacoat material PP 2038 Solution A and Solution B, applications were made to a fresh site. This was made necessary by reason of the fact that lesions resulting from the primary applications had not healed sufficiently to permit reapplication to the original site.

RESULTS

Material No. 1 - Polacoat material PP 2038 Solution A - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material No. 2 - Polacoat material PP 2038 Solution B - produced in all five rabbits a marked edema and erythema accompanied by vesiculation. This was apparent upon removal of the patches. These reactions were noted to have intensified during subsequent observations. At fourteen days following the primary application the lesions present precluded further use of the site. A similar response pattern was noted to the challenge application.

Material No. 3 - Lubricating oil, Weapons MIL-L-14107 - produced marked erythemas in all rabbits. These were noted upon removal of the patches. They had reduced in four of the rabbits at the 24-hour observation and had completely disappeared in all animals by the fourth day. A similar response pattern was noted to the challenge application.

Material No. 4 - Molybdenum disulfide, technical grade, MIL-M-7866A(ASG) - produced no reactions in any of the five rabbits to either the initial or challenge applications.

Material No. 5 - Polacoat material PP 2038 50/50 v/v Solution A and Solution B - produced marked erythema and edema accompanied by some vesiculation. This was apparent upon removal of the patches. No intensification of these reactions was noted during subsequent observations. At fourteen days following the primary applications the lesions present precluded further use of the site. A similar response pattern was noted to the challenge application.

CONCLUSIONS

Effects produced by Polacoat material PP 2038 Solution B and its 50/50 v/v admixture with Polacoat material PP 2038 Solution A were considered of sufficient severity to preclude further testing upon humans.

Reactions produced by the Lubricating oil, Weapons MIL-L-14107 were not considered to be of sufficient severity to preclude further testing in humans.

HUMAN PATCH TESTS

SHELANSKI REPEATED INSULT PATCH TEST

PROCEDURE

Each material was tested on three hundred human volunteer subjects. The sample was applied, using the conventional patch technique, to the skin of the subjects for twenty-four hours and then removed. Skin reactions were graded and recorded. The skin was allowed to recuperate for twenty-four hours. This cycle of contact and recuperation was repeated fifteen times for a total of thirty days, the reaction being graded after each application. Following the removal and the grading of the fifteenth application the skin was allowed to recuperate for two weeks. The material was then re-applied on the same subjects for twenty-four hours. Patches were then removed and the reactions were graded and recorded. The first application gave an index of primary irritation. The final application gave information on sensitization. The repeated applications, from the second through the fifteenth, determined the extent of fatiguing and served to accelerate skin reactions which facilitated forecasting of probability of cutaneous irritation due to long-term exposures.

RESULTS

Material No. 1 - Polacoat material PP 2038 Solution A. This material produced reactions ranging from mild to moderately severe in four subjects during the fifteen primary applications. The first of these reactions appeared following application seven, and increased irregularly in number during subsequent applications. Reactions to the final, or challenge, application were noted in three of the four subjects who had reacted earlier as well as in two subjects who had previously been non-reactive. The reactions noted would indicate that this material produced sensitization in five of the subjects tested.

Material No. 3 - Lubricating Oil, Weapons MIL-L-14107. This material produced reactions ranging from moderate to moderately severe in seven subjects during the fifteen primary applications. The reactions made their initial appearance in two subjects following the fourth application, increasing irregularly in number during subsequent applications. No reactions were noted as a result of the final, or challenge, application. The material thus appears to have some degree of fatiguing action which would tend to produce cutaneous irritation in long term exposure. It did not sensitize any of the subjects.

Material No. 4 - Molybdenum disulfide, technical grade, MIL-M-7866A(ASG). This material was not a primary irritant or a fatiguing agent to the 300 human volunteer subjects. This material did not sensitize any of the subjects.

CONCLUSIONS

In this study of five materials, only one, material No. 4 - Molybdenum disulfide, technical grade, MIL-M-7866A(ASG) produced no significant reactions by either the Schwartz prophetic patch test on rabbits (ref. 2) or the Shelanski repeated insult patch test on three hundred human volunteers (ref. 3). This material may be considered inocuous and may be permitted to contact human skin for prolonged periods. This conclusion is based upon a generally accepted testing procedure. However, it must be pointed out that the test method is not infallible or above criticism. Further, the patch test situation does not duplicate the range of temperature, humidity, air flow, perspiration, and friction, among other factors, which will be met in actual usage of the material. Because the prophetic patch test was devised to provide screening information with respect to cutaneous irritation and sensitivity from certain materials, it must be emphasized that the test should be used only for that purpose. The recommended procedure following the test is to employ the material within the limits recommended for direct skin contact on a usage basis. This should be done on 5,000 to 10,000 subjects, preferably under variable climatic conditions prior to the release of the material for general use.

With respect to the remaining four materials tested it is concluded that:

1. Polacoat material PP 2038 Solution B, due to its severe effect upon rabbit skin, is not safe to use in contact with the human skin.

2. Polacoat material PP 2038 Solution A and Polacoat material PP 2038 Solution B mixed in equal volumes, due to its severe effect upon rabbit skin, is not safe to use in contact with the human skin.

3. Polacoat material PP 2038 Solution A should not be considered for use in contact with the human skin due to possible sensitizing action.

4. Lubricating Oil, Weapons MIL-L-14107, because of its fatiguing action, is not considered safe for use in prolonged, repeated contact with human skin. Its use under conditions of short-term contact, with thorough washing following such contact, may be considered as relatively safe. The safety of such use should however be confirmed as outlined above.

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APPENDIX A

TABLE I

Maximum Intensity Of Reactions On Rabbit Skins

With

Polacoat Material PP 2038 Solution B

Lubricating Oil, Weapons MIL-L-14107

50/50 V/V Mixture Of Polacoat Material PP 2038 Solution A And Solution B

Material	Rabbit Number	Primary Application Day 1	2	3	4	Challenge Application Day 1	2	3	4
Polacoat Material PP 2038 Solution B	1	3+	4+	4+	4+	4+	4+	4+	4+
	2	3+	4+	4+	4+	3+	4+	4+	4+
	3	3+	4+	4+	4+	3+	4+	4+	4+
	4	3+	3+	4+	4+	3+	4+	4+	4+
	5	3+	3+	4+	4+	4+	4+	4+	4+
Lubricating Oil, Weapons MIL-L-14107	1	2+	1+	0	0	2+	1+	0	0
	2	2+	2+	1+	0	2+	1+	0	0
	3	2+	1+	0	0	2+	1+	0	0
	4	2+	1+	0	0	2+	1+	0	0
	5	2+	1+	0	0	2+	2+	0	0
50/50 V/V Mixture Of Polacoat Material PP 2038 Solution A And Solution B	1	3+	3+	3+	3+	3+	3+	3+	3+
	2	3+	3+	3+	3+	3+	3+	3+	3+
	3	3+	3+	3+	3+	3+	3+	3+	3+
	4	3+	3+	3+	3+	3+	3+	3+	3+
	5	3+	3+	3+	3+	3+	3+	3+	3+

APPENDIX B

TABLE II

Repeated Insult Patch Test

With

Polacoat Material PP 2038 Solution A

Number of subjects negative throughout	294
Number of subjects showing 1+ but no higher	0
Number of subjects showing 2+ but no higher	2
Number of subjects showing 3+ but no higher	4

Number of Application	Grade of Reactions				
	0	1+	2+	3+	4+
1	300	0	0	0	0
2	300	0	0	0	0
3	300	0	0	0	0
4	300	0	0	0	0
5	300	0	0	0	0
6	300	0	0	0	0
7	299	0	0	1	0
8	298	0	1	1	0
9	300	0	0	0	0
10	300	0	0	0	0
11	298	0	0	2	0
12	299	0	0	1	0
13	299	1	0	0	0
14	298	0	0	2	0
15	300	0	0	0	0
Sub Total	4,491	1	1	7	0
Challenge	295	0	2	3	0
TOTAL	4,786	1	3	10	0

APPENDIX B

TABLE III

Individual Scoring of Positive Reactions Produced by Polacoat Material PP 2038 Solution A

Subject Number	Exposure Number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Challenge
4	0	0	0	0	0	0	3+	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	3+	0	0	0	3+	0	0	0	3+
97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2+
98	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2+
110	0	0	0	0	0	0	0	0	0	0	3+	0	1+	3+	0	3+
259	0	0	0	0	0	0	0	2+	0	0	3+	0	0	3+	0	3+

Note: Underlined entries denote change of site of patch at next application.

APPENDIX B

TABLE IV

Repeated Insult Patch Test

With

Lubricating Oil, Weapons MIL-L-14107

Number of subjects negative throughout	293
Number of subjects showing 1+ but no higher	0
Number of subjects showing 2+ but no higher	4
Number of subjects showing 3+ but no higher	3

Number of Application	Grade of Reactions				
	0	1+	2+	3+	4+
1	300	0	0	0	0
2	300	0	0	0	0
3	300	0	0	0	0
4	298	0	2	0	0
5	300	0	0	0	0
6	300	0	0	0	0
7	300	0	0	0	0
8	299	0	1	0	0
9	298	0	1	1	0
10	300	0	0	0	0
11	300	0	0	0	0
12	300	0	0	0	0
13	300	0	0	0	0
14	297	0	0	3	0
15	300	0	0	0	0
Sub Total	4,492	0	4	4	0
Challenge	300	0	0	0	0
TOTAL	4,792	0	4	4	0

APPENDIX B

TABLE V

Individual Scoring of Positive Reactions Produced by Lubricating Oil, Weapons MIL-L-14107

Subject Number	Exposure Number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Challenge
65	0	0	0	0	0	0	0	0	2+	0	0	0	0	0	0	0
84	0	0	0	2+	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	2+	0	0	0	0	0	0	0	0	0	0	0	0
173	0	0	0	0	0	0	0	2+	0	0	0	0	0	0	0	0
226	0	0	0	0	0	0	0	0	0	0	0	0	0	3+	0	0
272	0	0	0	0	0	0	0	0	3+	0	0	0	0	3+	0	0
300	0	0	0	0	0	0	0	0	0	C	0	0	0	3+	0	0

Note: Underlined entries denote change of site of patch at next application.

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13. ABSTRACT

Five Air Force development materials were studied via the prophetic patch test method on laboratory animals to determine the primary irritant effect, gross sensitization index, and gross percutaneous toxicity of these materials. The patch test studies with rabbits indicated that two of the materials produced severe primary irritant action. A third material produced primary irritation whose severity was not considered sufficient to preclude testing in humans. Testing on human volunteers was therefore carried out with three of the materials. Results indicated that only one of these materials was safe to use in contact with human skin.

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